Recipe for capturing authentic embryonic stem cells may apply to any mammal, study suggests

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Researchers have what they think may be a basic recipe for capturing and maintaining indefinitely the most fundamental of embryonic stem cells from essentially any mammal, including cows, pigs and even humans. Two new studies reported in the December 26th issue of the journal *Cell*, a Cell Press publication, show that a cocktail first demonstrated to work in mice earlier this year, which includes inhibitory chemicals, also can be used to successfully isolate embryonic stem cells from rats.

Authentic rat embryonic stem cells had never before been established.

The new discovery made in labs at both the University of Edinburgh and the University of Southern California (USC), Los Angeles, is a major breakthrough for biomedical research, said Qi-Long Ying, an author on both studies who was at the University of Edinburgh and is now at USC. That's because it will allow researchers to readily produce genetically altered strains of rats, with conditions that mimic human disease, in a very targeted way. Austin Smith led the team at the University of Edinburgh and Ying led the USC team.

Humans and rats are physiologically more similar than humans and mice, making the study of rats more directly applicable to people, and rats' larger size also makes them easier to work with in many cases, according to the researchers. Humans and rats also tend to have similar responses

to drugs.

The findings lend support to the notion that embryonic stem cells will remain in their undifferentiated, pluripotent state when they are shielded from particular outside signals. (Pluripotent refers to the ability to differentiate into any cell or tissue type). Scientists had previously thought that the maintenance of stem cells depended on activating signals from outside, including growth factors and other chemicals.

Embryonic stem cells are derived from the inner cell mass of blastocysts. Blastocysts are hollow balls of cells that form in early development. The inner cell mass is a cluster of cells inside the blastocyst that goes on to form the embryo.

Authentic embryonic stem cells are defined by three cardinal properties: unlimited symmetrical self-renewal in the lab; comprehensive contribution to primary chimeras; and generation of functional egg and sperm for genome transmission. Chimeras are produced when embryonic stem cells are inserted into a developing blastocyst and those stem cells go on to contribute to a normal embryo with cells of two origins, Ying explained. Because those embryonic stem cells can contribute to the germ line, any genetic alterations they carry -such as the loss or gain of a gene--can be passed on to the next generation.

The versatility of embryonic stem cells, combined with the ease with which they can be manipulated genetically, has provided a powerful means to elucidate gene function and create disease models via the generation of transgenic, chimeric, and knock-out animals. Although embryonic stem cells have been routinely derived from particular strains of mice since 1981, their capture from rats or other animals had remained elusive.

While human embryonic stem cell lines do exist, Ying said, it's not clear

that they represent the most grounded stem cell state because the essential properties can't be demonstrated for obvious ethical reasons.

Now, Ying and Smith's teams show that a two- or three-ingredient concoction known as 2i or 3i respectively, which inhibits signals that would otherwise activate the differentiation process, maintains rat embryonic stem cells in their natural default state, allowing them to self-renew, or multiply, as generic stem cells. (The cocktails include inhibitors of GSK3, MEK, and FGF receptor tyrosine kinases.)

Most importantly, the isolated cells can produce high rates of chimerism when reintroduced into early stage embryos and can transmit through the germline, they report.

" In the past two decades, embryonic stem cells have been routinely used to create loss of function (knockout) or gene replacement (knockin) mutations by homologous recombination in the mouse, providing an invaluable tool for the functional characterization of genes," Ying's group wrote. "Now, the availability of true rat embryonic stem cells provides an opportunity to adapt the technology developed in the mouse to the rat."

The new findings raise "the possibility that culture formulations based on the 3i/2i principle could facilitate derivation of embryonic stem cells from other mammals, including livestock species," Austin Smith's team wrote. "It will also be of interest to investigate whether supernumerary human embryos cultured in 3i/2i may give rise to pluripotent cell lines that are qualitatively different from current human 'embryonic stem' cells" more like ground state rodent embryonic stem cells.

Source: Cell Press